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The Deterioration of Cotton Fabric by Certain Micro-Organisms

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INTRODUCTION

The deterioration of untreated tarpaulins, tentage, clothing, and other cellulosic articles by micro-organisms in warm, humid climates has always been great, but particularly during wartime has caused financial loss and decreased operational efficiency in the armed forces. To aid in solving some of the problems brought about by mildewing and rotting of materials, quantitative information is needed concerning micro-organisms that cause this deterioration. The purpose of this study was to determine the adaptability of a number of micro-organisms to a pure-culture technique and to evaluate their ability to deteriorate cotton fabrics.

Studies recorded in the literature (*7*, *12*, *13*, *18*)[2] of the growth of molds on cotton fabrics are, for the most part, qualitative, giving only names of the organisms found and sometimes a description of the appearance of the damaged material. Rogers, Wheeler, and Humfeld (*15*) gave a detailed review of the literature on the subject of micro-organisms on cotton materials, and for this reason no résumé of the qualitative work that has been reported is included in this circular.

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[1] Grateful acknowledgment is made to Charles Thom, Vera K. Charles, W. W. Diehl, and Harry Humfeld for identifying a number of the fungi used and to C. D. Sherbakoff for identifying the various species of *Fusarium*.

[2] Italic numbers in parentheses refer to Literature Cited, p. 11.

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Relatively few papers in the literature record work quantitatively measuring the damage caused in cotton fabric by pure cultures of fungi. In those studies reported, only a small number of species in but a few genera known to deteriorate cotton were used. Thom, Humfeld, and Holman (*19*) proposed a test employing *Chaetomium globosum* G. Kunze ex Fr. and a mineral-salts agar containing sodium nitrate for the nitrogen source as an added nutrient. These authors also studied the cotton-deteriorating ability of species of *Aspergillus*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Stachybotrys*, and *Trichoderma*, as well as that of several additional species of *Chaetomium* and the aerobic bacterium, *Spirochaeta cytophaga* Hutchinson and Clayton.[3] Several other workers have used the method of Thom, Humfeld, and Holman (*19*), with various modifications, in measuring fabric deterioration. Rogers, Wheeler, and Humfeld (*15*) studied the changes produced in cotton duck by *C. globosum* and by *S. cytophaga* in the presence of mineral-salts agar containing sodium nitrate. In studying the efficiency of different fabric finishes with respect to mildew resistance, Furry, Robinson, and Humfeld (*2*) used *C. globosum* as the test organism and sodium nitrate for the nitrogen source in mineral-salts agar.

The tendering caused in bleached cotton duck by *Alternaria* sp., *C. globosum*, *C. elatum* G. Kunze ex Fr., *Hormodendron* sp., *Metarrhizium* sp.,[4] and *Stachybotrys papyrogena* Sacc. was investigated by Greathouse, Klemme, and Barker (*5*), using sodium nitrate, ammonium nitrate, and ammonium dihydrogen phosphate as nitrogen sources in liquid mineral-salts media. Marsh, Greathouse, Bollenbacher, and Butler (*8*) studied *C. globosum*, *Metarrhizium* sp.,[5] *Aspergillus niger* v. Tiegh., and *Penicillium* sp. in evaluating copper soaps as rotproofing agents. To test with *C. globosum* they used mineral-salts agar containing sodium nitrate according to the procedure described by Rogers, Wheeler, and Humfeld (*15*), and for *Metarrhizium* sp. they followed the method of Greathouse, Klemme, and Barker (*5*), using a mineral-salts solution with ammonium nitrate for the nitrogen source. For *A. niger* and *Penicillium* sp. an ammonium nitrate medium supplemented with glucose and peptone was used. Greathouse and Ames (*4*) determined the deterioration of cotton duck by 16 species of *Chaetomium* in the presence of four mineral-salts media, using sodium nitrate, ammonium nitrate, ammonium dihydrogen phosphate, and ammonium sulfate as nitrogen sources. In determining the minimum protective dosage of certain fungicides on cotton fabric, Marsh, Greathouse, Butler, and Bollenbacher (*9*) used ammonium nitrate as the source of nitrogen in mineral-salts agar. The test fungi in this study were three species of *Aspergillus*, *C. globosum*, *M. glutinosum*, and one species each of *Hormodendron*, *Stemphylium*, and *Trichoderma*.

Furry and Zametkin (*3*), in their work on a soil-suspension method for testing mildew resistance, isolated in pure culture the organisms which grew from a soil inoculum on cotton fabric in the presence of a mineral-salts solution prepared with ammonium nitrate as the nitrogen source. They found that the action of *C. globosum*, *Metarrhizium* sp.[6] and one species of *Fusarium* resulted in complete deterioration of an 8-ounce cotton duck at the end of 14 days' incubation. In addition, the following fungi caused between 60 and 75 percent loss in strength of the cotton duck during the same period of incubation: *Acrostalagmus albus* Preuss, certain species of *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Papulospora*, and one species of *Penicillium*.

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[3] Stanier (*16*) proposed the name *Sporocytophaga myxococcoides* for *Spirochaeta cytophaga* Hutchinson and Clayton, which is the same organism Krzemieniewska called *Cytophaga myxococcoides*. Stanier does not consider this organism as synonymous with *Cytophaga hutchinsonii* Winogradsky.

[4] This organism was later recognized as *M. glutinosum* (11).

[5] See footnote 4.

Since comparatively little quantitative work has been done with different organisms which deteriorate cotton, this study was made of a number of cellulose-decomposing fungi isolated, for the most part, from cotton or cotton fabrics exposed to outdoor weathering or to soil.

Many of the fungi found on mildewed fabrics and the cellulose decomposers occurring in soil have a wide distribution over the surface of the earth, as is indicated by numerous records in the literature. On the other hand, some organisms that have been reported only occasionally may nevertheless be widely scattered. It is obviously impossible to obtain readily exact data upon the geographical distribution of these fungi. Bisby (*1*) has stated: "Mycologists have been able to map with accuracy the geographical distribution of comparatively few fungi . . ." The wide distribution of air-borne fungi has been reported by a number of authors. An excellent review of the literature on this subject is given by Morrow and Lowe (*10*). The predominating molds in these studies are much the same as those found on damaged cottons from the same locations.

MATERIALS AND METHODS

The test fabric used in this study was an 8-ounce bleached cotton duck. After being degreased with carbon tetrachloride and desized by enzyme treatment, the material was cut into strips 6 inches in the warp direction and 1¼ inches fillingwise. The strips were raveled to exactly 1 inch in width for breaking-strength measurements.

The method described by Greathouse, Klemme, and Barker (*5*) was used for testing the cellulose-decomposing abilities of the various fungi. In this procedure the 6-inch strips of cotton fabric, pulled down to 1 inch, were sterilized on glass-fabric wicks in square culture bottles containing liquid nutrient. The strips were then inoculated with a suspension of spores and mycelium of a pure, active culture of the fungus to be studied and incubated for 7 days in a controlled room at 85° F. and a relative humidity of over 90 percent. Ten replicate strips, selected at random, were used for each test.

After incubation the strips were washed, dried at a humidity below 65 percent, and conditioned overnight at 70° F. and 65 percent relative humidity. Breaking-strength determinations were then made by means of the Scott tester and the results were recorded as percentage loss for the average of the 10 replicate strips for each organism. The controls in these experiments consisted of sterilized, uninoculated strips which were incubated under the same conditions as the inoculated samples.

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[6] See footnote 4, p. 2.

The culture chambers were 16-ounce French square bottles with metal screw caps in which an area about 1 inch in diameter had been replaced by a filter of glass fabric to permit aeration of the samples during incubation.

The liquid medium was prepared by dissolving 1.3940 gm. K_2HPO_4, 0.7395 gm. $MgSO_4.7H_2O$, and 1.0006 gm. NH_4NO_3 in about 500 ml. distilled water and diluting to 1 liter. For some of the organisms two other media were also used, one in which sodium nitrate ($NaNO_3$) and the other in which ammonium dihydrogen phosphate ($NH_4H_2PO_4$) was substituted in equivalent quantities of nitrogen for the ammonium nitrate (NH_4NO_3) in the formula given above. Media supplying nitrogen in different combinations were tested since various bacteria and fungi are reported to have different nitrogen requirements (*14, 17*).

The organisms utilized were taken from stock cultures, most of which were isolated in the laboratories of the cooperating bureaus (table 1). Many of the fungi were obtained originally from mildewed cotton fibers, fabrics, and other cellulosic materials. Drawings illustrating certain species of some of the genera here used are presented as aids in recognizing them (figs. 1 and 2). All of the organisms in this work were fungi with the exception of *Spirochaeta cytophaga*, an aerobic bacterium which is thought to utilize cellulose exclusively as its source of carbon (*6*).

TABLE 1.—*Sources from which the micro-organisms used in this study were obtained*

Source	Organisms isolated [1]
Cotton boll	*Acremonium* sp. (1358.1). *Alternaria* sp. (1350.3). *Cephalosporium* sp. (1326.2). *Graphium* sp. (1332.1).
Cotton stem	*Cephalosporium* sp. (1359.1).
Raw cotton	*Alternaria* sp. (1022.2). *Cladosporium hebarum* (1343.1). *Fusarium equiseti* var. *bullatum* (1340.1). *F. moniliforme* (1004.1). *F. scirpi* (1004.2, 1004.3, 1004.7). *F. semitectum* (1004.8, 1004.9). *F. semitectum* var. *majus* (1004.4, 1004.6). *Hormodendron* sp. (316.1, 1236.1). *H. cladosporioides* (1229.2a, 1281.3). *Metarrhizium glutinosum* (1334.1). *Stachybotrys* sp. (1229.2b). *Stemphylium* sp. (1203.1).
Warehouse cotton	*Aspergillus* sp. (1335.2). *Metarrhizium glutinosum* (1334.2). *Trichoderma* sp. (1050.3).
Cotton in storage experiment	*Brachysporium* sp. (1052.1) *Chaetomium globosum* (1042.4) *Thielavia terricola* (1049.1, 1051.1) *Trichoderma* sp. (1050.1, 1050.2)
Cotton duck	*Aspergillus sydowi* (1335.1) *Stachybotrys papyrogena* (1331.1)
Cotton duck inoculated with soil	*Fusarium moniliforme* (1555, 1561) *Metarrhizium glutinosum* (1334.3)
Rotted fabric	*Stysanus medius* (1361.1)
Filter paper	*Stachybotrys atra* (1331.2)
Hibiscus	*Fusarium oxysporum* (1347.1)
	Aspergillus niger (TC215-4247) (from collection of Thom and Church)
	Penicillium luteum (1336.1)
Soil	*Spirochaeta cytophaga* (600)

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[1] The numbers after each name refer to the stock cultures of the organisms which were maintained for this study in the laboratories of the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering and of the Division of Textiles and Clothing, Bureau of Human Nutrition and Home Economics.

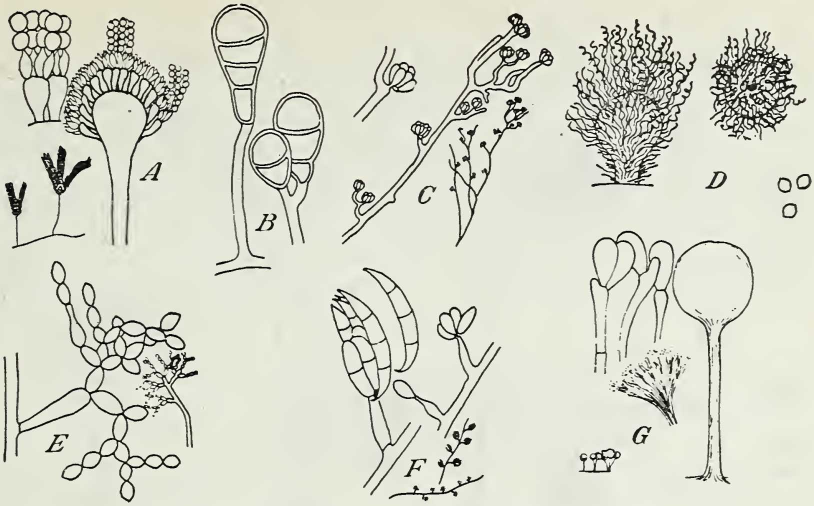

FIGURE 1.—Characteristic growth of fungi:

A, *Aspergillus*, growth at first white, usually changing with conidial production to various colors; texture cottony, velvety, or powdery. Conidia usually globose, borne in chains from flask-shaped structures (phialides) produced directly or secondarily upon the inflated end of the conidiophore.

B, *Brachysporium*, growth white at first, then brown, feltlike. Conidia brown, ovoid to pear-shaped, two- to many-celled and borne on simple conidiophores.

C, *Cephalosporium*, growth white, spreading and cottony. Conidia small, egg-shaped, colorless, borne in small heads on short, simple conidiophores.

D, *Chaetomium*, growth grey, velvety or feltlike texture. Conidia rarely seen. Ascomycetous fruiting bodies or perithecia globose, covered with straight or branched, and kinky or wirelike hairs. Ascospores brown, lemon-shaped, and produced within the perithecia.

E, *Cladosporium*, growth white, becoming grey to greenish-brown, cottony and spreading. Conidia greenish to tan, irregularly ovoid, one- or two-celled and borne in chains on variously branched conidiophores.

F, *Fusarium*, growth cottony, white, tending to stain the substrate various shades of red, purple, or blue. Conidia present in two forms, microconidia and macroconidia, the latter colorless, elongate, sickle-shaped, with two or more cells. Conidiophores colorless, short, simple, bearing waxy clusters of conidia.

G, *Graphium*, growth white, becoming dark as the fruiting bodies develop. Conidia produced on simple conidiophores as in *Cephalosporium* or in moist masses on stalks or coremia. Conidia small, egg-shaped, and colorless. Coremia dark, with globose apex.

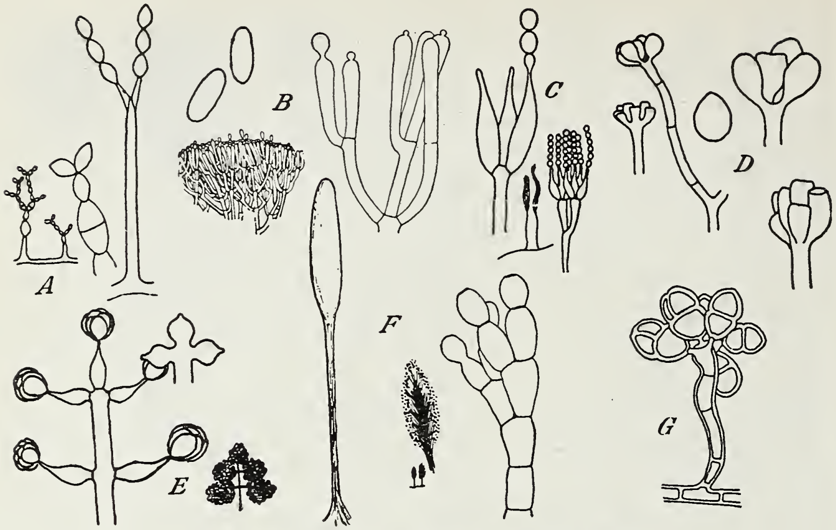

FIGURE 2.—Characteristic growth of fungi:

A, *Hormodendron*, growth green brown to brown, velvety to powdery. Conidia subglobose to ovoid, one-celled, green brown, and produced in chains, simple or branched, on branched conidiophores.

B, *Metarrhizium* glutinosum, growth feltlike and white, becoming dark green brown, moist and appressed in masses to the substrate as the conidia are formed. Conidia green brown, one-celled, elongate-ovate, borne on colorless branched, fingerlike conidiophores which are compacted into a palisade arrangement.

C, *Penicillium*, growth velvety or powdery, usually white, becoming yellow, green blue green, brown, buff orange, grey, or remaining white as the conidia are produced. Conidia one-celled, globose, produced in unbranched chains on cells (phialides) arranged in a penicillus or brushlike mass.

D, *Stachybotrys*, growth grey to black, appressed. Conidia ovoid, brown, and borne on inverted flask-shaped structures in clusters at the tips of long, simple conidiophores.

E, *Trichoderma*, growth white, commonly becoming green, usually appressed. Conidia globose, hyaline, in mass green or greenish brown, borne in clusters at the tips of conidiophores which are single or variously branched.

F, *Stysanus*, growth white, becoming grey. Conidia produced on branched conidiophores on dry powdery coremia. Conidia globose, colorless or slightly grey.

G, *Stemphylium*, growth feltlike, becoming velvety, greenish brown. Conidia divided into two or more cells by horizontal and vertical crosswalls. Conidiophores simple or branched, bearing clusters of dry brown conidia.

RESULTS AND DISCUSSION

A comparison of the cellulose-decomposing activities of the various fungi and of *Spirochaeta cytophaga* on cotton duck when incubated in the presence of ammonium nitrate as the nitrogen source, is given in figure 3. The results are expressed as percentage losses in breaking strength as compared with the average breaking strength of 141 pounds for the controls. In evaluating the data presented it must be borne in mind that the results are from only one set of conditions of temperature and humidity. Also there were relatively few variations made in the mineral-salts medium. The changing of any of these factors would probably alter the results.

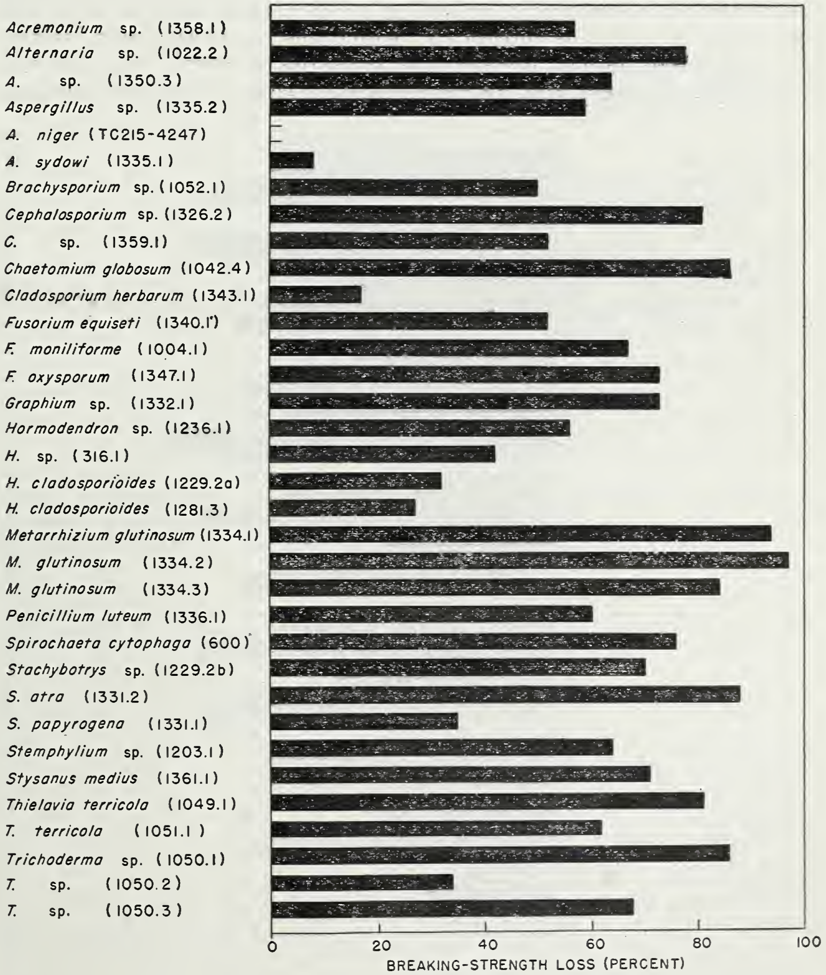

FIGURE 3.—Percentage loss in breaking strength caused by various micro-organisms grown on cotton duck on mineral-salts medium containing ammonium nitrate.

Of the 33 fungi included in figure 3, 24 caused over 50 percent loss in the breaking strength of the duck in the 7-day incubation period, and 8 of these tendered the fabric more than 80 percent. The greatest amount of deterioration (97 percent) was brought about by one of the strains of *Metarrhizium glutinosum* Pope (1334.2), while another isolate (1334.1) of the same genus produced the next highest loss (94 percent). *Stachybotrys atra* Cda., *Chaetomium globosum*, one of the isolates of *Trichoderma* (1050.1), *M. glutinosum* (1334.3), *Thielavia terricola* (Gilman and Abbott) Emmons (1049.1), and *Cephalosporium* sp. (1326.2) caused losses of 88, 86, 86, 84, 81, and 81 percent, respectively. Of the remaining 16 fungi that produced more than 50 percent loss in strength, 7 deteriorated the fabric in amounts ranging between 65 and 80 percent, and 9, between 50 and 65 percent. On the other hand some commonly occurring molds caused little or no tendering. *Cladosporium herbarum* Fr., which has frequently been isolated from mildewed fabric, lowered the breaking strength of the cotton duck only 17 percent and *Aspergillus niger* caused no deterioration.

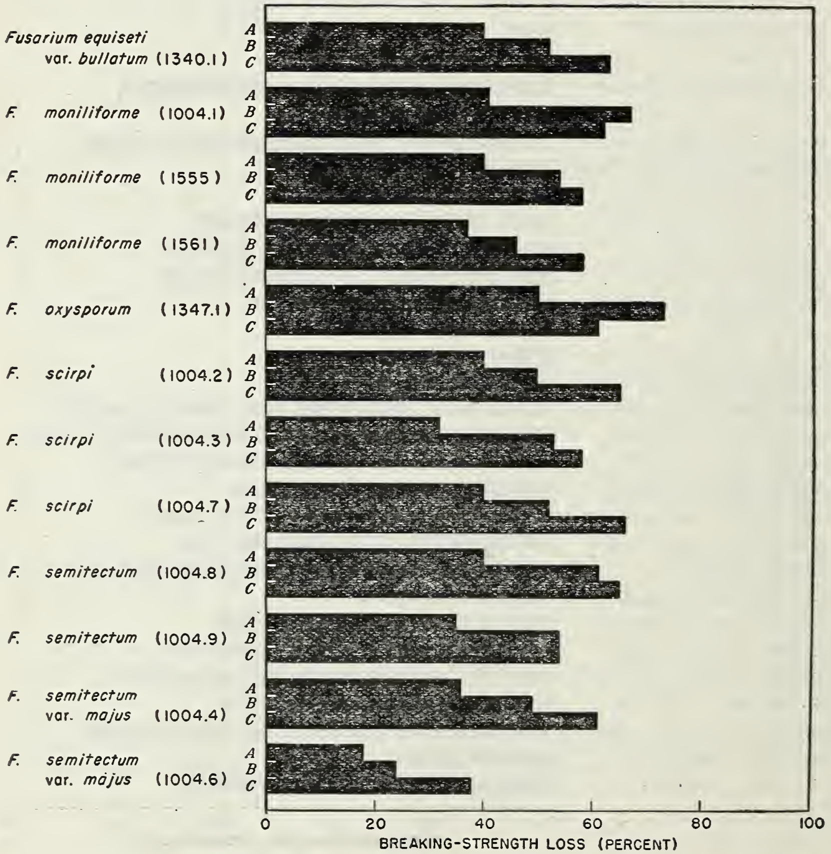

FIGURE 4.—Effect of nitrogen source on loss in breaking strength of cotton duck caused by various species of *Fusarium*. Nitrogen supplied as: *A*, sodium nitrate; *B*, ammonium nitrate; *C*, ammonium dihydrogen phosphate.

Spirochaeta cytophaga, the bacterium included in figure 3, produced 76 percent breakdown in the fabric in the presence of the mineral salts containing the ammonium nitrate.

Eight of the twelve *Fusarium* isolates (fig. 4) caused more than 50 percent loss in strength in the fabric on the ammonium nitrate medium. On this nutrient, *Fusarium oxysporum* reduced the fabric strength 73 percent. This was the greatest breaking-strength loss caused by any of the fusaria.

Large differences in cellulose-deteriorating ability are frequently found between species of the same genus and even between isolates of a single species. This fact is shown in figures 3, 4, and 5 wherever more than one species of a given genus was studied. The same phenomenon was also pointed out by Greathouse and Ames (*4*) in their study of various species of *Chaetomium.*

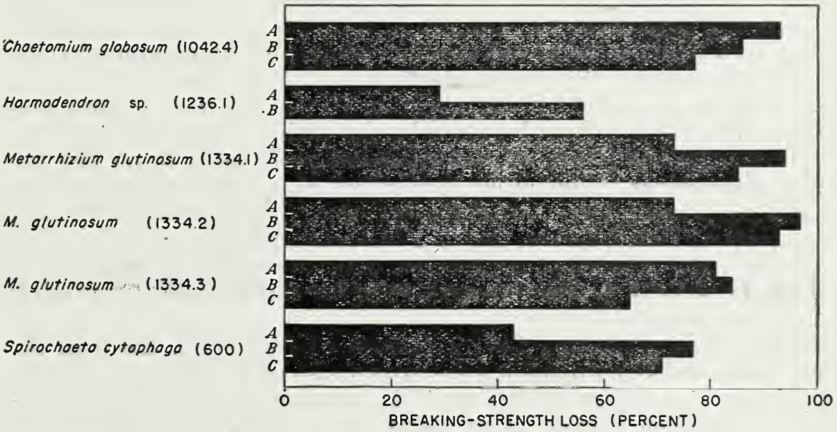

FIGURE 5.—Effect of nitrogen source on loss in breaking strength of cotton duck caused by different micro-organisms. Nitrogen supplied as: *A*, sodium nitrate; *B*, ammonium nitrate; *C*, ammonium dihydrogen phosphate.

The use of salts containing nitrogen in different combinations as nitrogen sources in the added nutrient influenced the amount of deterioration caused in the test fabric by a given organism (figs. 4 and 5). Ten of the 12 *Fusarium* isolates studied (fig. 4) were most destructive to the fabric when incubated on ammonium dihydrogen phosphate medium. The values obtained for these same fungi on the mineral-salts solution containing ammonium nitrate were, however, only slightly less than those obtained on the phosphate medium. The results in figure 5 show that the various organisms of other genera which were tested on more than one medium produced, in general, the most tendering of the fabric when the source of nitrogen was ammonium nitrate and the least when it was sodium nitrate. This was true for the three strains of *M. glutinosum*, *S. cytophaga*, and also for two of the *Fusarium* isolates—*F. moniliforme* (1004.1) and *F. oxysporum*, *Hormodendron* sp. (1236.1) was almost twice as destructive to the fabric on the ammonium nitrate as on the sodium nitrate medium but was not tested on the phosphate medium. *C. globosum*, however, caused the most deterioration of the cotton duck on the sodium nitrate medium and the least on the phosphate.

Some of the organisms studied in this work not only tendered the fabric but also stained it; others caused only deterioration. There are many fungi and bacteria that discolor or "mildew" materials but do not necessarily produce a loss in breaking strength. Also some fungi, such as *Aspergillus niger*, produce organic acids which may react with mildew- or rot-resistant treatments on fabrics, making these treatments ineffective (*8*). Staining of the fabric and effectiveness of mildew-resistant finishes were not considerations of this work. Therefore, with the exception of *A. niger*, organisms causing only discoloration or other reactions independent of cellulosic deterioration were not included.

SUMMARY AND CONCLUSION

Twenty-nine fungi out of the 43 isolates studied caused more than 50 percent loss in breaking strength of a cotton duck when incubated 7 days on a liquid mineral-salt medium containing ammonium nitrate as nitrogen source. The one bacterium used, *Spirochaeta cytophaga*, decreased the strength of the fabric by 76 percent on the same medium. The loss in strength caused by the various fungi ranged from 97 percent for one strain of *Metarrhizium glutinosum* to no loss for *Aspergillus niger*. The other strains of M. *glutinosum* produced 94 and 84 percent loss. *Stachybotrys atra*, *Chaetomium globosum*, one isolate of *Trichoderma*, one of *Thielavia terricola*, one of *Cephalosporium*, and *Fusarium oxysporum* deteriorated the fabric 88, 86, 86, 81, 81, and 73 percent, respectively, on the nutrient containing ammonium nitrate.

Chaetomium globosum, the 12 isolates of *Fusarium*, the 3 of *M. glutinosum* and *S. cytophaga* were grown also on mineral-salts media containing (1) sodium nitrate and (2) ammonium dihydrogen phosphate as nitrogen sources. One species of *Hormodendron* was grown on the sodium nitrate but not on the ammonium phosphate medium. In general, the *Fusarium* isolates produced the greatest amount of deterioration on the ammonium phosphate medium and the least on the sodium nitrate. The fungi belonging to the other genera and *S. cytophaga* caused more damage to the fabric on the ammonium nitrate nutrient than on the other media, except for *C. globosum*, which caused the greatest loss in breaking strength on the sodium nitrate medium.

The results of this study indicate that most of the organisms utilized show a specific relation in their nutritive reactions in respect to fabric deterioration and within these limitations are adaptable as test organisms.

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